THE ECOLOGY OF AN ITONIDID FLY ASSOCIATED WITH A RUST ON BACCHARIS PILULARIS CONSANGUINEA

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The gall midge under study herein has been named and described as a new species, *Clinodiplosis pucciniae,* by Dr. A. Earl Pritchard of the University of California (3). This fly was collected by Mr. J. W. Tilden in the course of work on the insect fauna of its host plant. The present writer engaged in a study of the life history of the midge before its identity as an undescribed species was determined by Dr. Pritchard, to whom specimens were sent for identification. Acknowledgments are due to Dr. Pritchard for his identification and description of the midge which have relieved the writer of the necessity of laboring over its taxonomy and to Professor Ferris of Stanford University for his direction through the course of this study.

The fly is associated with the fungus, *Puccinia evadens* Harkness, which is a rust that occurs on *Baccharis pilularis* subspecies *consanguinea* (deCandolle) C. B. Wolf. This evergreen shrub, sometimes called chaparral broom or coyote brush belongs to the family Asteraceae and occurs in some abundance over considerable areas of California.

Only the aecial stage of the rust bears an ecological relationship with this midge. It is the only stage of the rust which persists over a long enough period to provide an adequate and lasting food supply for the larvae of the midge.

LARVAL PERIOD

When the larvae hatch from the eggs, they are almost colorless. Those larvae hatching outside the aecia soon crawl into the spore mass. Here, as they begin to feed on the spores of the fungus, they assume the orange color of the spores.

Growth is very rapid immediately after hatching, providing the food supply is plentiful, the size increasing as much as three times within two days. In their early stages the larvae are quite elongated, but as they mature they seem to become proportionately shorter and wider.

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[1]The present study was completed at Stanford University.

Both the eggs and the young larvae are very susceptible to drying and when raised in captivity care must be taken to provide sufficient moisture. Usually a small bit of cotton or sand wet with a drop of water daily placed in the container being used for the larvae is sufficient to allay drying.

The larvae are most active in the spring, although the fall is an almost equally active period. Obviously the larval population is conditioned by the prevalence of the aecia whose numbers vary with the seasonal and climatic changes. In April an infested twig one half inch in diameter and one and a half inches long was observed to have fifty-seven larvae upon it. The eggs and larvae in various stages of development are found throughout the year. The smallest number of larvae is observed during the late summer months. The larvae which survive the dry season tend to become more sluggish and many build cocoons in preparation for the pupal stage. These cocoons are often built in the spore mass, in aecial pits which have dried out or on the surface of the soil. In captivity the larvae can be induced to build a cocoon between layers of paper toweling.

Building a cocoon is a fairly rapid process, requiring only one or two days. One larva was observed which completed its cocoon in the fold of a bit of paper toweling in only twenty-four hours. The cocoon is composed of minutely small silken threads, is arranged in a rather irregular network apparently with no set pattern, and is completely closed. The larvae secrete these threads from their mouths, probably a product of the salivary glands.

Though one commonly secures adults by raising them from specimens of the rust on which the larvae have built cocoons, the large number of larvae which fall from the fungus leads to the assumption that the building of cocoons may also occur in the soil. As mentioned above, larvae have been induced in the laboratory to spin cocoons in paper toweling. In their natural habitat it is quite conceivable that the larvae may build cocoons in the leaf mat below the plant. Furthermore, during the fall of the year, large numbers of eggs were found on the fungus, although close examination of numerous rust specimens revealed no cocoons. Inasmuch as the eggs are quite fragile and ordinarily hatch within five to eight days, it is evident that the adults, which live only a few days, had emerged currently from some place other than the fungus, probably from the soil. Rübsaamen (4) has found that some larvae of the closely related genus, *Mycodiplosis*,

pupate in the soil. Circumstances would seem to indicate that it is the exception rather than the rule for the larvae to pupate in the fungus. With specimens of larvae reared in the laboratory, a much greater percentage of the larvae fell out of the fungus than remained therein.

The larval stage is by far the longest stage in the life cycle of this species, sometimes lasting several months, in contrast to the two to four days of life as an adult midge, to the eight days of life as a pupa, and to the comparably short period of existence in the egg. Larvae were observed in the laboratory in cocoons on a twig, remaining without any apparent change in form from May until October.

A twig heavily parasitized by rust was collected on November 13. Examination under the microscope revealed that numerous eggs were present scattered in and around the aecia, the latter showing no evidence of being attacked by larvae. A further search of the twig revealed no larvae present. On the second day after collection numerous first instar larvae appeared. These immediately began to feed on the rust, growing very rapidly and causing a great number of white, empty spore cases to collect above the aecia. The larvae continued to attack the rust vigorously until November 25 at which time their food supply showed signs of being depleted. Many of the larvae had grown to full size by this time and many had left the twig, falling to the bottom of the plugged phial in which the twig was contained. On November 26 two pupae, not enclosed in a cocoon, were found on the bottom of the phial. The adults emerged eight days later. Allowing five to seven days for the eggs to hatch and three days as adults, the life cycle required approximately thirty-two days. This, however, took place in the laboratory where the atmospheric factors were favorable and the food supply was plentiful. In less favorable circumstances one would expect the cycle to take longer, as evidenced by the long larval period during the summer months cited above.

The generations apparently overlap, creating a situation wherein there are always specimens present in various degrees of development. At least this seems to be the case in this region where the winters are mild and the summers are not excessively dry and hot. In a region where the winters are quite severe, one would expect larvae (inside their cocoons) to undergo a resting "winter

stage." In his studies of British Itonididae, Barnes (1) found that emergence of the adults was retarded by cold weather and that the larvae remained in the cocoons until spring.

NUTRITION

Larvae in all stages of development are often found with their heads buried far down into the aecia, suggesting that they might well take their nutrition from the juices of the rust mycelium as well as from the rust spores. This, however, was not definitely determined. Aecia have been observed which have been completely devoured within seven to eight days by the feeding of from six to ten larvae in each.

Due to the small size and structure of the mouthparts of the larvae the fungal spores cannot be ingested but are sucked dry of their contents. Spores were never found in the intestinal track of the larvae, though globules of orange oil similar to those contained within the spores were found. These globules of oil became readily visible if the larvae and also the spores were boiled in a potassium hydroxide solution. Since the spore walls are not broken down by this treatment, they would appear, if present, within the body wall of the larvae. Grasse (2) found larvae of *Mycodiplosis reaumuri* Kieffer and *Mycodiplosis tremulae* Kieffer living on the underside of leaves infested with fungi. He states that the larvae pierce the spore envelope with fine sylets and then suck out the contents. With *Clinodiplosis pucciniae* Pritchard the great accumulation of white, empty spores in the vicinity of the working larvae serves as evidence that the spores are not eaten but that their contents are merely sucked out.

METHOD OF LOCOMOTION

The method of movement of a larva was observed as it crossed a smooth glass surface. Forward motion was brought about by issuing forth a thin silky thread from the tip of the mouth parts. The thread was attached or stuck to the glass surface. The body of the larva was then contracted with a peristaltic-like motion, drawing the posterior end forward. The posterior end is capable of sticking to the glass surface and with an elongation of the body the anterior portion is again thrust forward whereupon the thread is reattached at a new forward position. In this way the larvae are capable of a relatively rapid movement even on a smooth

surface. This means of locomotion even permits the larva to scale a vertical surface. One larva two milimeters in length was observed to move over a paper surface at the rate of two centimeters per minute.

Larvae have also been observed in locomotion without employing the thread. In the natural habitat locomotion is further facilitated by bristles situated over the body surface which aid the organism in acquiring a hold on the substrate. In addition the mouth parts form a projection with which the larva can anchor itself into irregularities in the substrate and pull itself forward.

The silky web secreted by the larva is readily visible in the infested aecia of *Puccinia evadens* Harkness. The web causes the shed spores and empty spore envelopes to be held together in a mat covering the fresher, still attached spores beneath. This condition is disadvantageous to the rust in that it allows fewer aeciospores to be disseminated. On the other hand the mat o spores provides protection for the larvae, which are usually embedded in the aecia beneath it, both from excessive drying and from the possibility of becoming the prey of birds or large predacious insects.

PUPA

The general structure of the pupa does not differ greatly from that of other Itonididae. It is worthy of note, however, that a pair of strong, cephalic bristles are inserted in the region between the eye position of the pupa. These are to be distinguished from the so-called "cephalic horns" which are located just anterior to the insertion of the antennal rudiments and which are not particularly produced in this species. The bristles probably function in the penetration of the cocoon wall by the pupa. A number of empty puparia was found which were protruding from the cocoon with only the tip of the abdominal portion remaining embedded in the wall of the cocoon. Apparently the pupa breaks out of the cocoon just prior to the emergence of the adult.

A female midge was observed emerging from its puparium. The puparium splits longitudinally along the dorsum in the head region. The head of the insect emerges first followed by the thorax, the wings, the abdomen, the antennae and last the legs. Immediately after emergence the legs appeared to be quite incapable of supporting the body of the imago. However, within a few minutes the chitin in the legs appeared to have become firm

enough to provide adequate support. The wings were wrinkled and folded on emergence, but rapidly expanded to their normal size. The insect maintained a horizontal resting position while expanding the wings and the expansion of the wing seemed to accompany a series of convulsive movements of the thorax near the wing attachment. The length of time required for complete emergence amounted to slightly less than five minutes.

The eyes, wings, antennae, and legs become a dark gray before emergence from the puparium. The color of the thorax and abdomen is a pronounced orange immediately after emergence, but this gradually darkens to a gray color within a few hours.

ADULT

The length of time spent in the adult stage is very short. Imagoes reared from larvae in the laboratory were never observed to live over three days. They do not appear to be especially strong flyers. In the laboratory female midges did not lay eggs when they were not fertilized. The mouth parts of this species are of the type which can take up only liquid substances. An adult of this species was observed taking up droplets of water which had condensed on the surface of the glass phial in which it was contained. No further observations were made, however, in regard to the nutrition of the adult flies.

EGG

The eggs of this species of *Clinodiplosis* are ellipsoidal and almost transparent, having a very pale orange color. The length is approximately twice the width of the egg and the ends are of equal size and curvature. The adult female deposits them in the aecia and among the spores which have been shed and which lie around the periphery of the aecia. The chorion of the egg is covered with a quite sticky substance at the time of deposition, causing the egg to adhere to the substrate and also causing the aeciospores to adhere to the egg surface. This condition makes the egg inconspicuous and difficult to distinguish from the rest of the spore mass.

The length of time required for the eggs to hatch seems to be rather irregular. Most required from five to seven days to hatch. Eight to twelve eggs are often found placed singly in no set arrangement around an aecium.

No evidence of paedogenesis was found in this species. The larger eggs characteristic of paedogenesis were not found and

neither did larvae isolated over a long period of time ever produce eggs.

SYMBIOSIS BETWEEN FLY AND FUNGUS

The helation of the fly to the fungus has already been indicated in the discussion of the life history of the former. It may, however, be summarized as follows:

1. On the basis of all present information the fly occurs only in association with this particular fungus.

2. In its active growing period the larva of the fly lives only in the aecia of the fungus and feeds upon the aeciospores. Apparently no association exists with the other fungal stages.

3. Examination of stomach contents of fly larvae indicates that they pierce the fungus spores and ingest only the contents of the spores.

4. There is no indication that the adult flies make any use of the fungus spores as food.

5. There is no indication that the flies have anything to do with the dissemination of the fungus, since no fungus spores were found clinging to the bodies of flies.

6. The larvae eat so many of the spores, at times cleaning out almost an entire pustule, that they probably have some effect in reducing the total number of spores available for dissemination.

7. The webs spun by the larvae seem also to have a retarding effect upon spore dissemination by preventing the spores from being shed readily.

8. Thus, it appears that the relation of the fly and the fungus is quite simple, the fly feeding upon the fungus and apparently being restricted to it for its food supply, but the fungus receiving no aid in any way from the fly and being in no way dependent upon it.

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